



Applicant: Bernard John Carroll
U.S. Serial No.: 09/701,926
Filing Date: June 1, 2001

Docket No.: 111590-120

IN THE SPECIFICATION

The Examiner has stated that an abstract of the disclosure is required on a separate sheet for this application. Accordingly, an Abstract of the Disclosure is provided herewith on a separate sheet, in compliance with this requirement.

On page 1, please replace the paragraph at lines 24-30 to page 2, line 1, as follows:

The subject specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (e.g. <400>1, <400>2, etc.), which nomenclature is synonymous for, and used herein interchangeably with, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, etc.

On page 5, please replace the paragraph at lines 11-20, as follows:

PMGSs may or may not be closely related at the nucleotide sequence level although they are closely functionally related in modulating phenotypic expression. Particularly preferred PMGSs are represented in <400>1; <400>2; <400>3; <400>4; <400>5; <400>6; <400>7; <400>8; <400>9; <400>10; <400>11; <400>12; <400>13; <400>14;

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<400>15; <400>16; <400>17; <400>18; <400>19; <400>20; <400>21; <400>22;
<400>23; <400>24; <400>25; <400>26; <400>27; <400>28; <400>29; <400>30; and/or
<400>31 SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ
ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11;
SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID
NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22;
SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID
NO:28; SEQ ID NO:29; SEQ ID NO:30; and/or SEQ ID NO:31 as well as nucleotide
sequences having at least about 25% similarity to any one of these sequences after
optimal alignment with another sequence of a sequence capable of hybridizing to any
one of these sequences under low stringency conditions at 42°C.

Please replace the paragraph on page 6, lines 17-26, as follows:

Accordingly, another aspect of the present invention provides a PMGS comprising the
nucleotide sequence:

<400>1; <400>2; <400>3; <400>4; <400>5; <400>6; <400>7; <400>8;
<400>9; <400>10; <400>11; <400>12; <400>13; <400>14; <400>15;
<400>16; <400>17; <400>18; <400>19; <400>20; <400>21; <400>22;
<400>23; <400>24; <400>25; <400>26; <400>27; <400>28; <400>29;
<400>30; and/or <400>31 SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3;
SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8;
SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID
NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17;
SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID
NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26;
SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; and/or

SEQ ID NO:31; or a sequence having at least about 25% similarity after optimal alignment of said sequence to any one of the above sequences capable of hybridizing to any one of the above sequences under low stringency conditions at 42°C.

Please replace the paragraph on page 16, lines 5-8, as follows:

The present invention is further directed to the putative *Dem* promoter and its derivatives. The *Dem* promoter is approximately 700 bases in length extending upstream from the ATG start site. The nucleotide positions of putative *Dem* promoter are nucleotide 3388 to 4096 (Figure 5). The nucleotide sequence of the *Dem* promoter is set forth in <400>8 SEQ ID NO:8.

Please replace the paragraph on page 20, lines 27-29, as follows:

Figure 4 is a representation showing a sequence comparison between the potato α -amylase promoter (15) <400>2 SEQ ID NO:2 and the tomato α -amylase promoter <400>1 SEQ ID NO:1. The location of the UQ406 insertion is shown.

Please replace the paragraph on page 20, line 31 bridging page 21, lines 1-6, as follows:

Figure 5 is a representation of a nucleotide sequence <400>3 SEQ ID NO:3 of tomato genomic DNA from 651 bp upstream of the *Ds* insertion (acttcgag: underlined) in UQ406 to the beginning of the *Dem* coding sequence, followed by the *Dem* cDNA sequence from the ATG start site at base pair 4097 (sequence underlined). The target sequences of the *Ds* insertion in UQ406 and *Dem* ATG are underlined. The *Dem* cDNA sequence is shown in italics and underlined. The putative *Dem* promoter begins at nucleotide 3388 and ends just immediately prior to the ATG, i.e. at position 4096 <400>8 SEQ ID NO:8.

Please replace Table 1 at pages 24-26, as follows:

TABLE 1

SUMMARY OF SEQUENCE (SEQ) IDENTIFIERS

SEQ IDENTIFIER	DESCRIPTION
<400>1 <u>SEQ ID NO:1</u>	Nucleotide sequence of tomato α -amylase gene promoter
<400>2 <u>SEQ ID NO:2</u>	Nucleotide sequence of potato α -amylase gene promoter
<400>3 <u>SEQ ID NO:3</u>	Nucleotide sequence of genomic DNA upstream of <i>Dem</i> gene followed by <i>Dem</i> cDNA coding sequence in tomato line UQ406
<400>4 <u>SEQ ID NO:4</u>	Nucleotide sequence upstream of <i>Ds</i> insertion (i.e. upstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato line UQ12
<400>5 <u>SEQ ID NO:5</u>	Nucleotide sequence downstream of <i>Ds</i> insertion (i.e. downstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato line UQ12
<400>6 <u>SEQ ID NO:6</u>	Nucleotide sequence of portion of putative tomato (UQ12) homologue of potato patatin gene
<400>7 <u>SEQ ID NO:7</u>	Nucleotide sequence of portion of potato patatin gene having homology to <400>6 <u>SEQ ID NO:6</u>
<400>8 <u>SEQ ID NO:8</u>	Nucleotide sequence of putative <i>Dem</i> promoter in UQ406
<400>9 <u>SEQ ID NO:9</u>	Nucleotide sequence upstream of <i>Ds</i> insertion in tomato mutant UQ11

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- <400>10 SEQ ID NO:10 Putative PMGS from UQ11 corresponding to nucleotides 1 to 295 of <400>9-SEQ ID NO:9
- <400>11 SEQ ID NO:11 Putative PMGS from UQ11 corresponding to nucleotide
- <400>12 SEQ ID NO:12 Nucleotide sequence of an upstream portion of putative sucrose synthase gene in tomato (UQ14) containing PMGS
- <400>13 SEQ ID NO:13 Nucleotide sequence of an downstream portion of putative sucrose synthase gene in tomato (UQ14) containing PMGS
- <400>14 SEQ ID NO:14 Putative PMGS from UQ14
- <400>15 SEQ ID NO:15 Partial nucleotide sequence of 3' untranslated region from potato sucrose synthase
- <400>16 SEQ ID NO:16 PMGS from UQ14
- <400>17 SEQ ID NO:17 Partial nucleotide sequence of 3' untranslated region from potato sucrose synthase
- <400>18 SEQ ID NO:18 PMGS from UQ14
- <400>19 SEQ ID NO:19 Partial nucleotide sequence of 3' untranslated region from potato lactate dehydrogenase (LDH)
- <400>20 SEQ ID NO:20 PMGS from UQ14
- <400>21 SEQ ID NO:21 Partial nucleotide sequence of intron II of tomato phytochrome B1 (PHYB1)
- <400>22 SEQ ID NO:22 PMGS from UQ14
- <400>23 SEQ ID NO:23 Partial nucleotide sequence of 3' untranslated region from potato sucrose synthase
- <400>24 SEQ ID NO:24 PMGS from UQ14
- <400>25 SEQ ID NO:25 Partial nucleotide sequence of 3' untranslated region of potato lactate dehydrogenase (LDH)

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<400>26 <u>SEQ ID NO:26</u>	PMGS from UQ14
<400>27 <u>SEQ ID NO:27</u>	Partial nucleotide sequence of intron I of potato cytosolic pyruvate kinase (CPK)
<400>28 <u>SEQ ID NO:28</u>	PMGS from UQ14
<400>29 <u>SEQ ID NO:29</u>	Partial nucleotide sequence downstream of <i>Brassica napus</i> 1.7S seed storage protein, napin (<i>napA</i>)
<400>30 <u>SEQ ID NO:30</u>	PMGS from UQ14
<400>31 <u>SEQ ID NO:31</u>	Partial nucleotide sequence of 3' untranslated region of tomato chorismate synthase 2 precursor gene (CSP)
<400>32 <u>SEQ ID NO:32</u>	Nucleotide sequence of an upstream portion of <i>Ds</i> insert containing PMGS in tomato (line UQ13)
<400>33 <u>SEQ ID NO:33</u>	Nucleotide sequence of an downstream portion of <i>Ds</i> insert containing PMGS in tomato (line UQ13)
<400>34 <u>SEQ ID NO:34</u>	PMGS from UQ13
<400>35 <u>SEQ ID NO:35</u>	Partial nucleotide sequence of tomato expansin 2
<400>36 <u>SEQ ID NO:36</u>	PMGS from UQ13
<400>37 <u>SEQ ID NO:37</u>	Partial nucleotide sequence of tomato ADP-glucose pyrophosphorylase
<400>38 <u>SEQ ID NO:38</u>	PMGS from UQ12
<400>39 <u>SEQ ID NO:39</u>	Partial nucleotide sequence of tomato Ca ²⁺ ATPase

Please replace the paragraph at page 28, lines 12-21, as follows:

GenomeWalker (14) is used to clone the tomato DNA sequences flanking the *Ds* element in UQ406. The DNA flanking the *Ds* element in line UQ406 is cloned and sequenced, and a search of the PROSITE database reveals that the *Ds* has inserted into the promoter region of an α -amylase gene. The promoter ~~<400>~~¹ SEQ ID NO:1 shows strong similarity to an α -amylase promoter of potato (15; Figure 4) ~~<400>~~² SEQ ID NO:2 and the coding sequence of the gene has strong homology with one of 3 reported potato α -amylase cDNAs (16). The DNA from 651 bp upstream of the UQ406 insertion to the end of the *Dem* coding sequence, has been sequenced (Figure 5) ~~<400>~~³ SEQ ID NO:3. Other such sequences have been located and cloned (see below) using the method of Example 4. Nucleotide sequences disclosed herein which flank the active *nos:BAR* gene are designated "phenotype modulating genetic sequences" or "PMGSs".

Please replace the paragraph at page 30, lines 15-35 bridging page 31, lines 1-2, as follows:

The sequence upstream of the *Ds* insertion (i.e. upstream of the *nos:Bar* gene) is as follows:

AATCAAAGAG	GAATTNAATT	CCNCAAAATT	TCATCCATAG	ATTTTGNGTC	50
TCTGAAAATT	AAAGTGACTT	TGTAATCTGA	AACCTAGAGT	CCTCAACCAT	100
ATCATTGACC	ATTAAGCCAT	ACCCTTAAAT	GTAGGGAATT	TGAAGTTTTA	150
AAAACCACAC	TTTGTTATTT	ATTGGCCCAA	ATACTCGATA	ATCTTTACAT	200
TATTGAAAAT	CAACATTCAA	AAGGAACGAA	CCTTCAATCA	CACCATCAAT	250
GTCAACTTTC	TTTTATTTTG	GATAATCTAA	GTTTTTAAAT	TGCAGTAAAA	300
TNAAATAAAA	CCCTAAACTT	CTTCTAGGTT	GAGACTTAGT	AAATATGAAT	350
TATATAAAGA	ATTCATGACA	AATGAGACAT	AAGAATAGTG	CCAGCAAATT	400
ACTTTTTTGA	TATCTTATCT	GTGATATCGG	AATTTTAACT	ACCATAAATT	450
TATGAATGAA	ATATCACTTA	TCTATTAGAG	AGGATTTAAT	CTCCCTTATA	500

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ATGACATTGA TAAAAGCAAG NACAAGTGCT CTTTATTTCT TAATTACAAA 550
TCCTTAAATA GATAAAAGCT ACGAATAACA TAATATCCTT AAATAGATAA 600
AAGCTACGAA TAACATAATA GTATATTACT CCNAATTATT TTGATTTATT 650
TAAAATGACT CCACTAATCC TGATGTGGTC TAGG <400>4 SEQ ID NO:4 684
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Please replace the paragraph at page 31, lines 4-20, as follows:

The tomato sequence immediately downstream of the *Ds* insertion (i.e. downstream of the *nos:BAR* gene) is as follows:

```
GGTCTAGGCC CTGGGTCTAG GAAACAAAAT AACTTATTTG ACTCCTAAAC 50
AATAGCAACA TACAAACCAC TGATATTGTA CAAGTAAAAT TCAATAAAAT 100
TCTAGCTCTC TCAAAACACTT TTAAAATTGT TATTTCTGTT TTGTCTGTGT 150
CATATTATGA CCTACACAAC AACAACAACA ACGAATTAG TGAAACTCTA 200
CAAAGTGGAG CCTGAAGTCG AGAGTTTACG CGGGCCTTAT CACTATCTTT 250
TCGAGATAAA AAAATTATTT TTAAAAGATC ATCGACTTAA ACAAACCAAA 300
CAATAATTAA AAAAATATGA ATTAATAGCA AAGCAGTGTG GACCATATAT 350
ACAAAAATCT ATAACAACAA CAAGGTGCAG AGCATTATTC CAACTAAGAT 400
CGAAGTTGTG ATACTGTCAT AATAAAAAATG ACACATATTT TGACAACATA 450
AAAAATAAAT AACCATAAAA TATATCATAG AAAAATGAAT ATATTAGAAC 500
AGCTCACTCC AATATTAAAA GAGAGAAAAA AAATATTTTC CCACCACAAT 550
GCCATAATCC TTGAGCTTAG CTATTTATAA GTAAAAAATA TGTTTTCTTG 600
GATAAATAGA AAAAGAAATA ATAATTAAAC ATAACCAATC ACTTCACAAA 650
TAAGAGTCTA TT <400>5 SEQ ID NO:5 662
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Please replace the paragraph at page 31, lines 22-29, as follows:

The level of homology between the potato and a tomato sequence is as follows:

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Tomato: 307 ATTTATTTT TAGGAAAAATTATCTAAATACACATCTTATTTTACCATATACTCTAAAAAT 248
      | ||| ||||| ||||| ||||| ||| | ||| ||||| |
Potato: 1914 AATTATATT TAGGAAAAATTACATAAATACACAACCTTAATATATTATTTCTCTAAAAAT 1973
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247 TCC 245    <400>6 SEQ ID NO:6  
    |||  
1974 TCC 1976  <400>7 SEQ ID NO:7
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Please replace the paragraph on page 32, lines 16-28, as follows:

A mutant tomato plant designed UQ11, was subject to characterization. The UQ11 *Ds* insertion resulted from transposition of the *Ds* back into the T-DNA, but it is slightly closer to the right border and in the opposite orientation (Figure 13). Figure 12 shows the DNA sequence upstream of the UQ11 *Ds* insertion. Nucleotide 1 is the first nucleotide upstream of the *Ds* (and the active *nos:BAR* gene). The sequence for nucleotides 1 to 295 is T-DNA sequence corresponding to the right border of tomato transformant 1561E (5), the starting position of the *Ds* before lodging in the *Dem* locus. This is nucleotide sequence <400>10 SEQ ID NO:10. Nucleotides 296 to 886 (in italics) [~~<400>11~~ SEQ ID NO:11] correspond to tomato genomic DNA flanking the T-DNA insertion in 1561E. Note the *Bam*HI/*Bcl*I fusion sequence (TGATCC) and the *Hpa*I site (GTTAAC), both in bold in the Figure 12, immediately upstream of the insertion site (see Figure 1). The putative PMGSs of UQ11 reside in the right border of the T-DNA (nucleotide 1 to 295), and/or the flanking tomato DNA (nucleotide 296 to 886). Another PMGS may also be located further upstream.

Please replace the paragraph on page 32, lines 33-34 bridging page 33, line 1, as follows:

A *Ds* insertion mutant, UQ14, resulted in *nos:BAR* expression. The transposon had, therefore, inserted proximal to a PMGS. The nucleotide sequences comprising PMGSs are represented in <400>12 SEQ ID NO:12 and <400>13 SEQ ID NO:13.

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Please replace the paragraph on page 33, lines 3-8, as follows:

A series of comparisons between ~~<400>~~12 SEQ ID NO:12 and other genes or nucleotide sequences was conducted:

- (1) Homology between PMGS-UQ14 sequence [~~<400>~~14 SEQ ID NO:14] upstream of *Ds* insertion and the 3' untranslated region of a potato sucrose synthase (*susi*) gene, Acc. no. AF067860 (70% homologous over about 200 bp):

Please replace the paragraph on page 38, lines 20-26, as follows:

Plasmid pUQ505 or pUQ511 were used as the starting vectors for constructing expression vectors containing putative PMGSs for bioassay. Tomato sequences flanking the reactivated *nos:Bar* insertions of UQ406, UQ11 and UQ14 were inserted into pUQ505 at the *NotI* site and into pUQ511 at either the *NotI* site or the *EcoRI* site or both. For example, pUQ505 was partially digested with *NotI* and the putative 886 bp-PMGS from UQ11, as shown in ~~<400>~~9 SEQ ID NO:9, was ligated into the new *NotI* site (formed as described above), in both orientations, to generate pUQ527 and pUQ5211 (Figure 7).

Please replace the paragraph on page 39, lines 24-31, as follows:

Surprisingly, DNA sequence analysis shows that the *Ds* insertion in UQ406 is located only about 3 kb upstream from the ATG of the *Dem* (Defective embryo and meristems) gene which has been cloned by tagging with *Ds* (Example 4). In fact, only about 700 bp of DNA separates the putative α -amylase STOP codon and the *Dem* ATG codon (Figure 8). This region presumably contains the promoter of the *Dem* locus and its nucleotide sequence is shown in ~~<400>~~8 SEQ ID NO:8. The *Dem* gene is required for correct patterning in all of the major sites of differentiation, namely in the embryo, meristems, and

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organ primordia. The function of *Dem* was determined by STD, somatic tagging of *Dem*.
Figure 8 provides a diagrammatic representation of the STD genotype. Mutant *dem+7*